

EFFECTS OF REPETITION AND DARK ADAPTATION
ON VISUAL EVOKED RESPONSES IN THE RAT

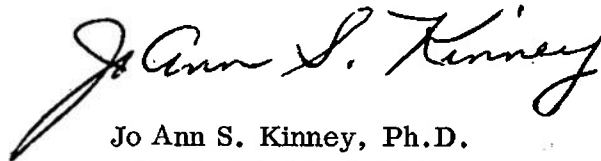
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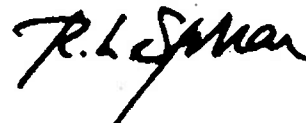
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SUMMARY PAGE

THE PROBLEM

To determine the changes occurring in the averaged evoked cortical response of rats when repetitive visual stimuli are used, so that such effects can be taken into account or utilized in studies of the neural effects of hyperbaric environments.

FINDINGS

No consistent changes were observed in the early components, providing the rats were first dark-adapted. However, certain changes did occur in the amplitude of the rats' later averaged evoked response components which are best explained in terms of central nervous system habituation.

APPLICATION

The results of this experiment can now be taken into account in hyperbaric experiments in which the averaged evoked response is used as a measure of neural functioning. Furthermore, the specific changes that were related to habituation effects on the surface can easily be compared with those that might occur in hyperbaric environments, to assess the effect of pressure and gas mixture on this elementary neuro-behavioral process.

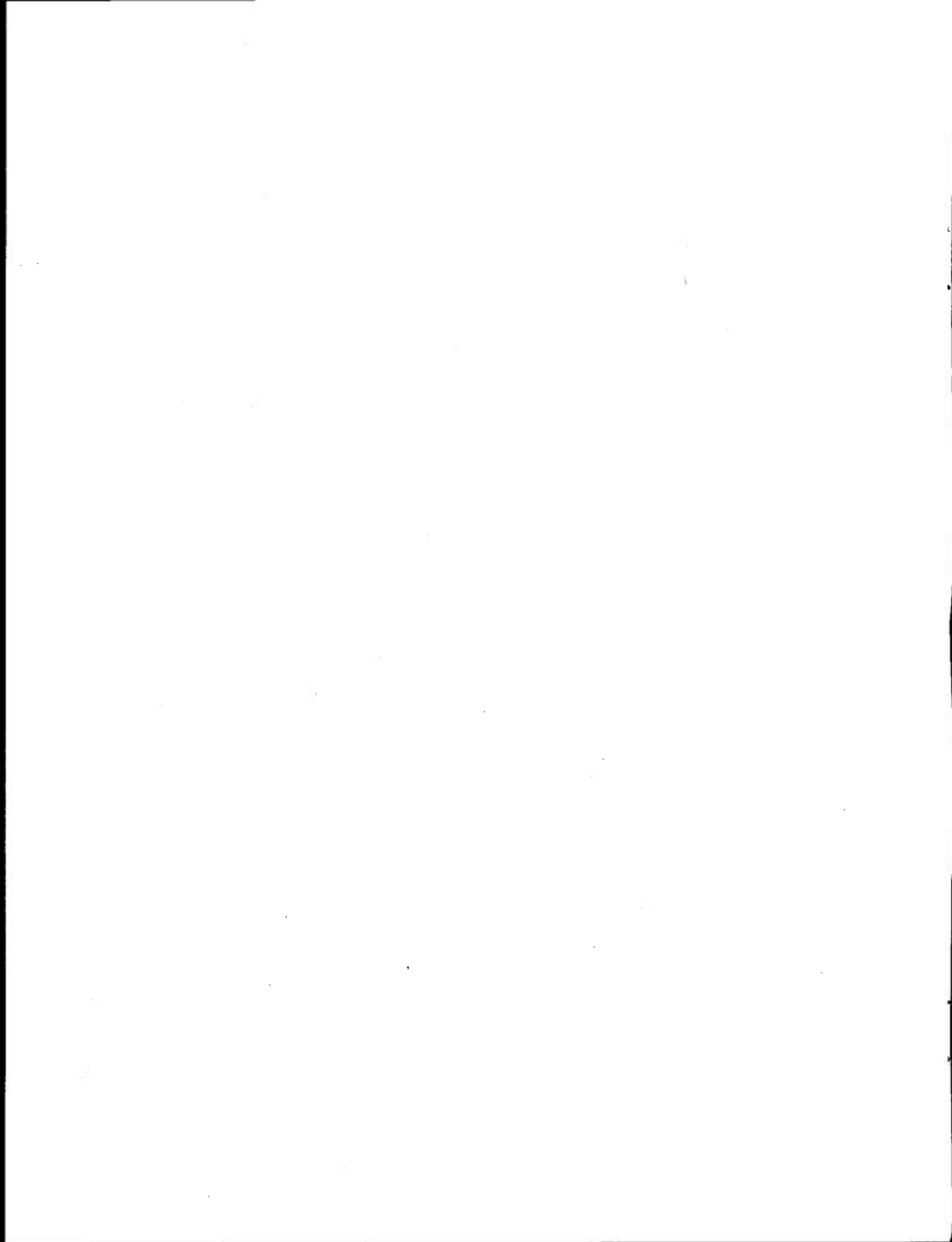
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ABSTRACT

Rats chronically implanted with monopolar electrodes in the primary visual cortex were used to evaluate progressive changes occurring in the amplitude of visual evoked responses to repeated visual stimuli. It was found that if the rats were dark-adapted prior to testing, no consistent changes occurred in the early components of the evoked response. Without sufficient dark-adaptation, however, progressive increases in these components were observed. Contrary to these changes, the later evoked response components exhibited reliable changes over trial blocks, regardless of whether prior dark adaptation was given. It was concluded that these later-component changes are best explained as correlates of central nervous system habituation. These data may be taken into account in hyperbaric experiments in which the averaged evoked response is used as a measure of neural functioning, thus often alleviating the need for certain costly control conditions. Also, they may provide an additional tool for research programs interested in assessing the effects of hyperbaric environments on simple neurobehavioral processes.



EFFECTS OF REPETITION AND DARK ADAPTATION ON VISUAL EVOKED RESPONSES IN THE RAT

INTRODUCTION

The development of sophisticated signal-averaging techniques has led to an increase in the use of sensory-evoked responses in the study of basic brain functioning. This technique has recently spread to hyperbaric research and has been successfully applied to the evaluation of neural dysfunction associated with nitrogen narcosis;¹⁻⁷ the averaged neural responses evoked by repetitive stimulation, such as flashing lights, have been compared at various pressure levels.

One possible shortcoming for its use in hyperbaric research, however, is that changes in the averaged evoked responses may result from the repetitive nature of the stimulation. Changes resulting from sensory habituation, for example, may contribute to the undefined variability of the data and lead to quite erroneous conclusions. This possibility therefore requires special controls, such as the substitution of helium for nitrogen in the gas mixture so that the effects specific to nitrogen narcosis can be identified. However, the use of such controls is quite inefficient both in money and time, and so from a practical standpoint, it is important to know what changes in the averaged evoked response are simply a function of the technique.

A more theoretical consideration, interesting in its own right, rests on the premise that habituation is one of the most elementary forms of behavior

in both animals and man.* It is believed to be directly related not only to the manner by which sensory information is processed, but many contend that it is also a basic form of behavioral plasticity, directly related to learning mechanisms in general.⁸ Thus, if certain changes in the components of evoked responses could be shown to be correlated with the process of habituation, this information would not only enhance our knowledge of the process, but also it would provide a potential tool for studying the effects of nitrogen narcosis on elementary behavioral processes.

In fact, one popular approach adopted in an effort to determine the underlying mechanisms responsible for habituation has been to record evoked responses during repeated sensory stimulation trials in an attempt to isolate the particular neural changes that occur. Although a cursory review of this literature suggests that the evoked response recorded in the sensory pathways exhibits significant decrements in amplitude with repeated stimulation,^{9,10} a closer examination reveals that the evidence is inconsistent (for critical reviews, see Thompson,¹¹ Thompson & Spencer⁸). Further, some of the investigators who have reported decreases in the amplitude of the sensory evoked response have been criticized for lack of adequate controls or statistical evaluation.

**For the purpose of this paper, the conventional definition of habituation is accepted, in which it is presumed that the phenomenon is mediated by the central nervous system.*

Several authors have pointed out a variety of possible confounding artifacts. For example, Guzman-Flores, Alcaraz, and Harmony¹², and Fernandez-Guardiola, Roldan, Fanjul, and Castells¹³ demonstrated that decreases in amplitude may sometimes result from peripheral influences, such as inner ear muscle contraction or pupillary constriction, while Marsh, Worden and Hicks¹⁴ found that significant changes in evoked response amplitude occur with rather subtle changes in the subject's body position. Also, Worden and Marsh¹⁵ demonstrated that unless stringent statistical procedures are used when evaluating changes in evoked response amplitude, incorrect conclusions may be drawn about chance effects. Thus, the attenuation that all of these authors observed was not due to actual changes in neural responsiveness to the stimulation, but rather to other less interesting, confounding variables.

Thus, the second purpose of this study was aimed at gaining a clearer understanding of how habituation actually occurs by a technique designed to separate its effects from purely peripheral ones. To this end, the effects of successive trains of flashing lights were determined, together with the effects of variations in two other variables which should influence evoked responses — stimulus intensity and dark adaptation. To establish the experimental control required, the study was made on restrained, cortically implanted rats.

METHOD

Subjects and Surgery

Sixteen male albino rats (Charles River CD strain) weighing approximately 600 grams were used in the experiment. Details of the chronic electrode implantation technique have been previously described by Ferris and Bartus.¹⁶ Briefly, miniature, self-tapping stainless steel screws (size 00, 1/8" long) were used as monopolar, epidural electrodes and were placed over the rat's primary visual cortex (3 mm lateral and 2 mm anterior to the lambda). The reference and ground screws were positioned near the ipsilateral and contralateral frontal sinuses, respectively, 3 mm lateral and 5 mm anterior to the bregma (see Fig. 1). These screw-electrodes were connected to a miniature Amphenol strip-connector socket using teflon-coated stainless steel wire. The screws and socket assembly were then embedded in a single mound of dental acrylic. A recovery period of at least two weeks elapsed before the rats were used in the experiment.

Apparatus

To obtain visual evoked responses (VERs), the unanesthetized rat was placed in a totally dark room, shielded from 60 cycle noise and high frequency interference. Gross body movements were restricted by placing the rat in a clear Plexiglas restraining device (see



Fig. 1. Screw-electrodes threaded into rat's skull, and positioned over visual cortex and nasal sinuses.

Ferris & Bartus¹⁶). A large rectangular box (11" x 17" x 11" high) with a semi-gloss silver interior was placed over the restrainer. The stimuli for eliciting the VERs were produced by placing the light source from a Grass PS-2 photo stimulator over an opening in the top of this box, directly above the rat's head. The rat's electroencephalogram (EEG) was amplified with a Grass P511 pre-amplifier and the VERs were obtained using a Computer of Average Transients (Technical Measurement Corp.). Each VER was computed on-line from a total of 50 individual one second sweeps and was immediately printed out using an x, y plotter.

Procedure

The stimuli were discrete flashes of light once every second from the photo-stimulator at one of two different stimulus intensity levels. The brighter

intensity was produced by setting the photo-stimulator intensity level at "8". The dimmer stimulus was produced by setting the level at "1" and using a 1.0 neutral density filter to further reduce the intensity by 90 per cent. Thus, these parameters produced two intensity levels which differed by almost two log units.

The effects of two different levels of dark adaptation were also studied. In the "no-dark-adaptation" condition, the rats were placed in the darkened experimental room for about three minutes before the testing session was begun. In the "dark-adaptation" condition, an interval of 30 to 35 minutes of total darkness elapsed before testing was initiated. Four rats were assigned to each of the four treatment combinations, so that each rat was run at only one intensity level and only one state of dark adaptation. The experimental session consisted of obtaining 10

successive VERs, with 90 seconds elapsing between each set of 50 flashes.

In the data analysis, the amplitudes of the main components (see Fig. 2) were measured, with the amplitude defined as the peak to peak deflection from the previous component. The data for the ten trials were blocked into five successive pairs. Using the amplitudes for trial block 1 as the baseline, the amplitudes for the four remaining blocks were expressed as a percentage change from that baseline. The percentage change data were subjected to a four-way analysis of variance, with components and trial blocks as repeated

measures factors. Subsequent three-way analyses were carried out for each VER component.

RESULTS

The complete statistical analyses which were carried out revealed a number of meaningful and statistically significant interactions among the variables which were analyzed (p 's $< .05$ or less). The major findings are illustrated in Figs. 3 and 4. The rat's state of dark-adaptation affected only the first three VER components, N1, P1, and N2. As shown in Fig. 3, the amplitudes of these

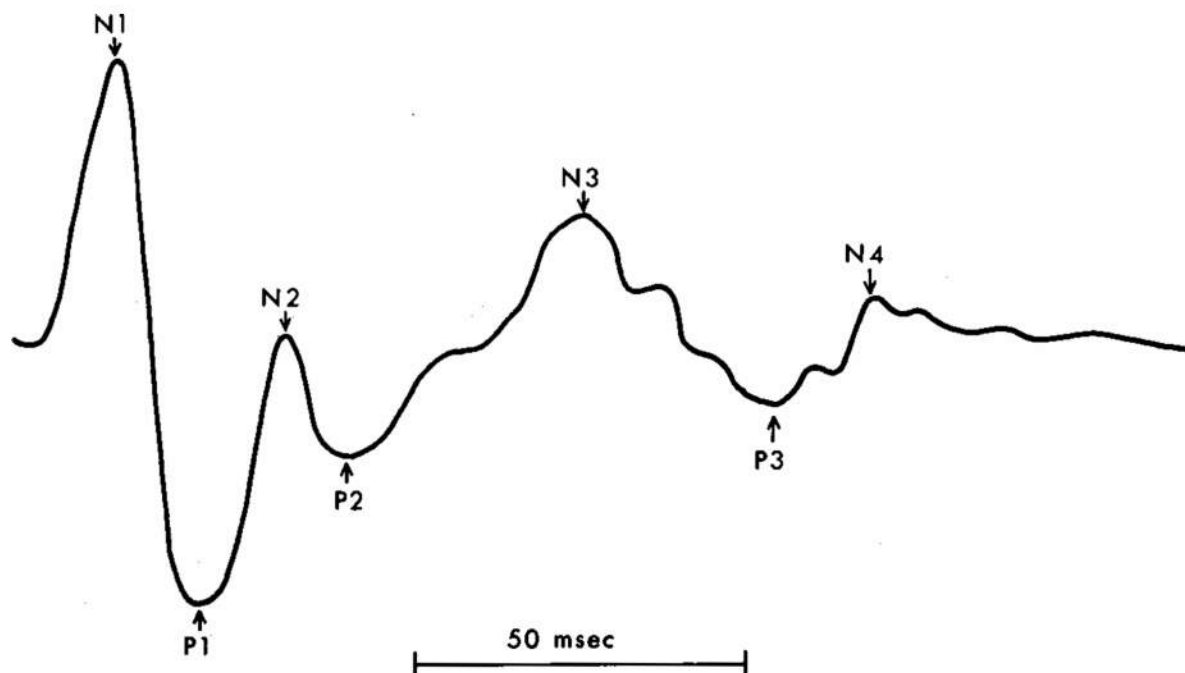


Fig. 2. Typical VER recorded from primary visual cortex. The major components which were analyzed are descriptively labeled, with negative polarity shown as upward deflection.

however, these components gradually increased in amplitude during the test session, continuing to grow 25 min. after testing was initiated. It is evident from Fig. 3 that the greatest increases

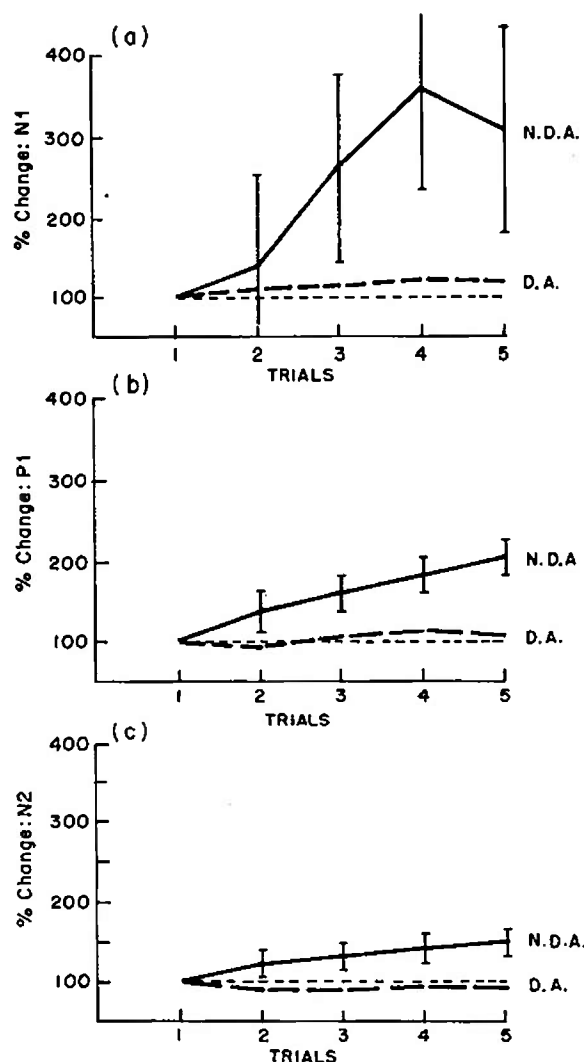


Fig. 3. % changes in the early components of the dark adapted (D.A.) and non-dark-adapted (N.D.A.) rats. The data were blocked from 2 successive trains of 50 flashes to obtain the five trial points shown. Vertical lines represent 0.05 critical difference scores computed from the ANOVA.

components did not change over successive trial blocks when the animals were dark adapted. Without dark-adaptation,

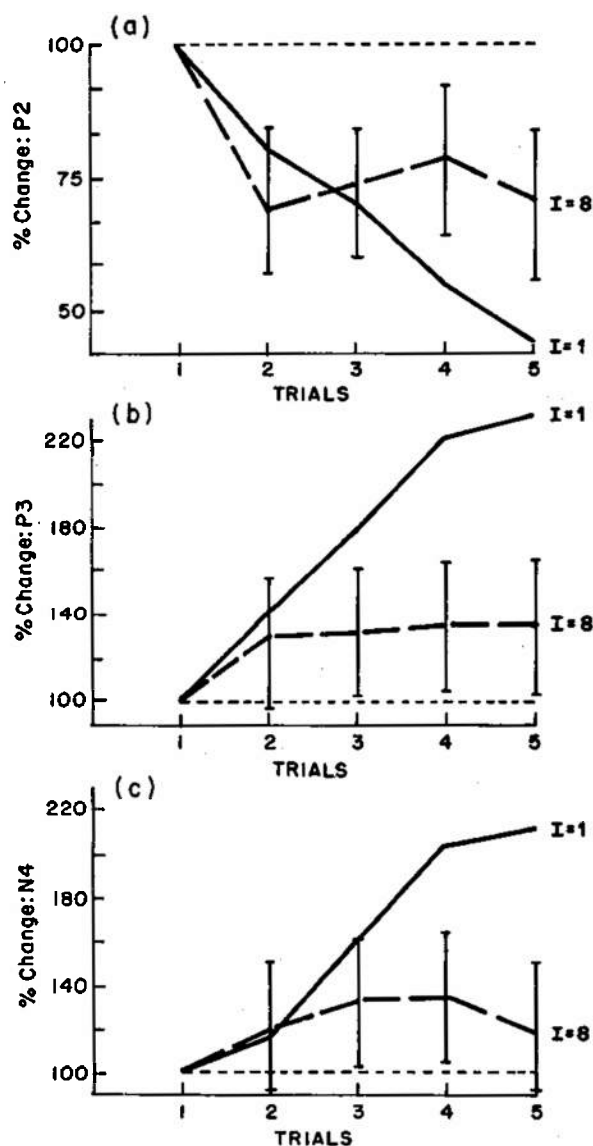


Fig. 4. % changes in later components for the two intensity levels. No effect of dark adaptation occurred for these components. Trial points were obtained as described in Fig. 3. Vertical lines represent 0.05 critical difference scores computed from the ANOVA.

occurred in the first component (N1), with progressively less of an increase occurring in P1 and N2. Another result which is not shown in Fig. 3 is that the increases in amplitude that resulted from a lack of dark-adaptation were greater for the low intensity flash than for the high intensity.

The changes in the later VER components (illustrated in Fig. 4) were not affected by the state of dark-adaptation. The amplitude of P2 decreased over successive trial blocks, while the amplitudes of P3 and N4 increased, and the amplitude of N3 (not shown) did not change. Furthermore, the magnitude of the changes which occurred in these later components was highly dependent on the flash intensity. At the low intensity, the changes were large and continued to increase with each successive trial block. At the high intensity, however, the changes were relatively small, and little, if any, further change occurred after trial block 2.

DISCUSSION

The results of this study clearly show that consistent changes do occur in the amplitude of the rat's visual evoked response over the course of repeated stimulation. However, those changes which might be considered to be the result of habituation were absent in the early part of the VER. Only rats that were not dark-adapted showed changes in the first three components while those rats which were dark-adapted showed no changes in these components over successive trials. The changes that were observed in the non-dark-adapted rats, supplemented

by the finding that greatest increases occurred with the less intense stimulation, are interpreted as demonstrating significant increases in the amplitude of the early VER components as the stimuli become subjectively more intense. That is, as dark-adaptation gradually progresses, the apparent brightness of the flashing light should progressively increase, with the dimmer stimulus showing ever greater apparent increases due to progressively greater availability of unbleached rhodopsin. That dark-adaptation influenced only the early VER components is consistent with the view that the earlier portion of the VER is primarily correlated with variables related to physical parameters of the sensory stimulus (John^{17,18}). Also interesting, and consistent with this view is the fact that the very first component was most influenced by dark-adaptation, with each successive component showing a progressively smaller effect (see Fig. 3).

These results indicate that changes in the level of dark-adaptation, which were sometimes still influential after 25 minutes in total darkness, provide yet another potentially confounding variable which must be controlled when changes in the amplitude of the VER are being studied. Recent evaluations of cortical and subcortical VERs from dark-adapted and non-dark-adapted cats indicate that this phenomenon is not peculiar to VERs obtained from the cortex of the albino rat (Bartus, unpublished observations, NSMRL, Groton, Conn.). At the same time, the present data indicate that as long as sufficient dark-adaptation is given, no confounding changes can be expected to occur in the early evoked response components over

successive trials, when hyperbaric tests are run.

Unlike the changes in the early components of the rat's VER, the later components were not at all influenced by the degree of dark-adaptation. Nevertheless, significant changes with repeated trials did occur, and an obvious question concerns whether these changes represent true central habituation effects. Two alternate possibilities have been a major source of criticism of past studies and must therefore be contended with here. One suggestion is that the effects are due to peripheral influences such as pupillary contraction and changes in receptor orientation due to head movement. The other suggestion is that the effects are the result of simple receptor fatigue. The experimental procedure was designed to minimize both of these possibilities, and a close look at the data suggests that these effects were in fact negligible. The use of the albino rat, whose unpigmented iris has little light absorption capabilities, and an experimental arrangement which has proven to provide consistent VERs from trial to trial (see Ferris & Bartus¹⁶) hopefully minimized influences from peripheral changes.

Since no changes occurred in the early VER components (providing sufficient dark adaptation was given) and the late component changes that did occur to repeated stimulation were manifested as increases as well as decreases (dependent on the particular component), any influence due to consistent peripheral changes can be effectively ruled out. On the other hand, it was hoped that receptor fatigue could be elimi-

nated by arranging the experimental procedure so that the intertrial interval was of long enough duration to enable the retina to recover adequately from the repeated stimulation of each individual trial. This attempt was also apparently successful, for no decreases occurred in the early components, as would be predicted, and the decrease that did occur in the late components was greater for the less intense stimulus. This is, of course, exactly the opposite of what would be expected on the basis of receptor fatigue and is perfectly consistent with the behavioral habituation data of Groves & Thompson¹⁹. For these reasons, it is concluded that the changes that occurred in the late components of the VER to repeated stimulation are accounted for most easily by the concept of sensory habituation.

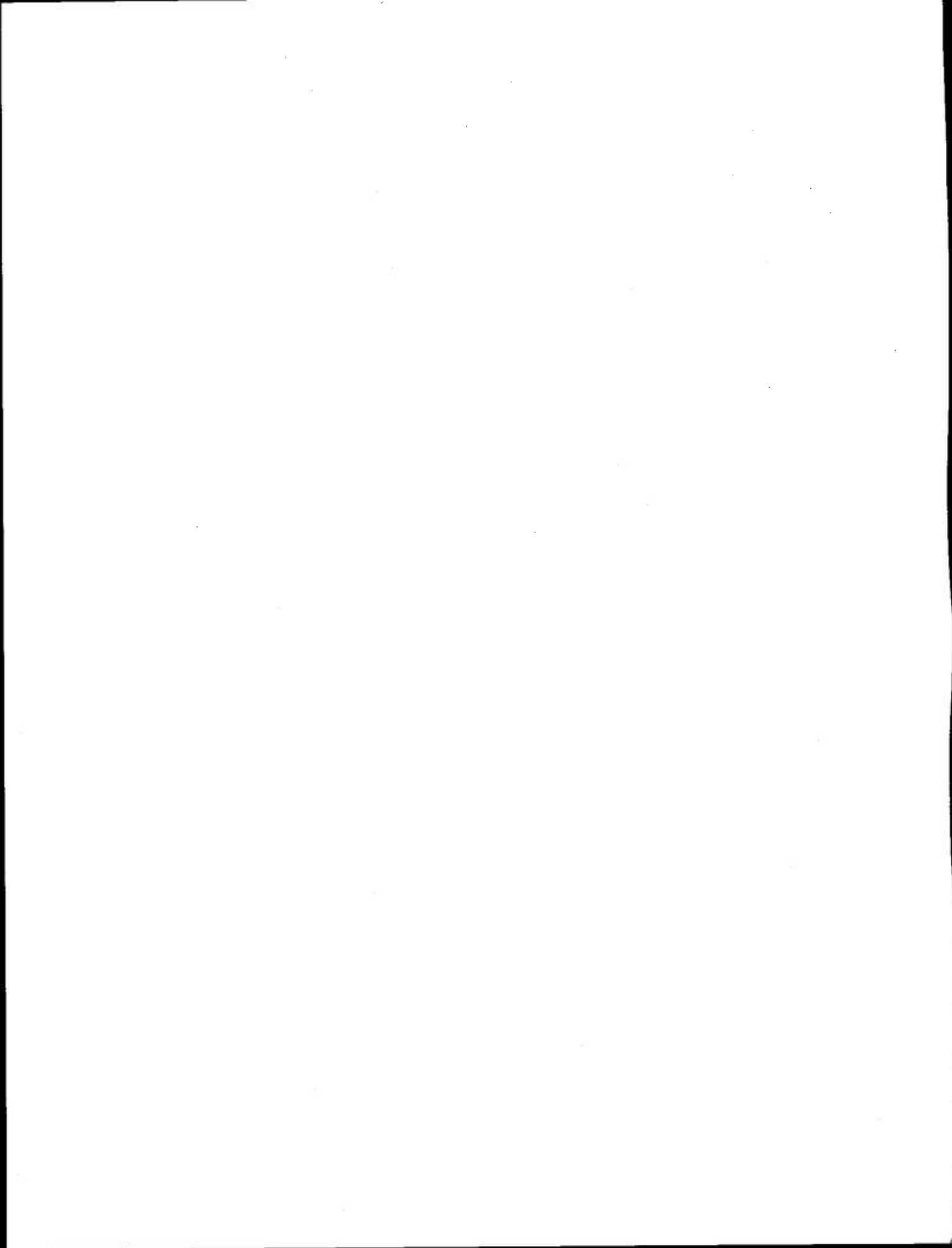
In conclusion, it was demonstrated first, that in an adequately dark-adapted rat no significant changes occur in the amplitude of the early VER components over successive trials. Thus, any changes that do occur in these early components during hyperbaric dives can presumably be attributed to the effects of pressure and gas mixture on neurosensory mechanisms. Second, consistent changes in the later components were found that are correlated with habituation. Not only does this demonstrate that neural activity in the sensory system is in some way altered during the course of habituation, but it is also a potentially powerful tool for investigating the deleterious effects of nitrogen narcosis on elementary behavioral processes. Such information is of obvious significance for the design of future behavioral hyperbaric research studies.

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